

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: **B6;129S5-*Angptl4*Gt(OST352973)Lex/Mmucd** MMRRC: **032147-UCD**

Protocol:

Reagent/Constituent	Volume (μL)
Water	10.775
10x Buffer	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1. (stock concentration is 20μM)	0.5
Primer 2. (stock concentration is 20μM)	0.5
Primer 3. (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	25.000 μL

Comments on protocol:

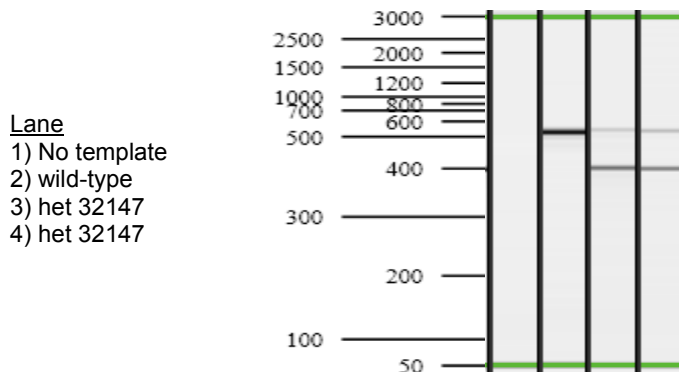
- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')	Electrophoresis Protocol:		
1. 32147-ComF	GCAAGATCCAGCAATTGTTCCAGAAGG	Argarose: 1.5% V: 90		
2. 32147-wtR	ACCCTTGATGAGAGCCTAGTGAGC	Estimated Running Time: 90 min.		
3. 32147-MutR	GGTGCCCCAAGGACCTGAAATGACC	Primer Combination	Band (bp)	Genotype
		1 and 2	496	WT
		2 and 3	383	Mut



Lexicon Contract Name: DNA467
LexVision Name: SEC270T1
Genentech ID: UNQ171



Mouse Accession(s): AF317839
Omnibank Clone: OST352973, VICTR48

Mutation Description:

Listed below is a portion of the mouse genomic sequence ([from mouse chr.17](#)) surrounding the gene trap insertion site in the Omnibank mouse line SEC270T1 (OST352973). The sequence below includes 250 nucleotides of sequence on either side of the gene trap insertion site, which is denoted with an asterisk *.

```
5'  
AGGACCTGGGTGGAGAAGGGCCTCAGAGCAGACTGTGAAAGGGTAGAAGGGAGGGTGAATAAGAGGAGGT  
TGCCGACATAAAGCCAGGTCTTCACTAGCCTTGCATATCTTCAGACTCAGCTCAAGGCTCAAAACAGCAA  
GATCCAGCAATTGTTCCAGAAGGTGGCCCAGCAGCAGAGATACCTATCAAAGCAGAATCTGAGAATACAG  
AATCTTCAGAGCCAGGTAATTACCCAGCAATTGGCCAA*ATGGGAAAGAGGAAACAAAGTGGGGGTGA  
AATGTAGTGATGAAATGTGGCTAAGCAGTTGTTATGGCATGAGTCAAAAGATCTAACCACCCGTTTCCTG  
CCCGGTCTTAGATAGACCTCTTGGCCCCACGCACCTAGACAATGGAGTAGACAAGACTTCGAGGGGAAA  
GAGGCTTCCAAGATGACCCAGCTCATTGGCTTGACTCCCAACGCCACCCACTTACACAGTGAGTATCTC TGGTCTTTGCT  
3'
```

Mutation Illustration:

Accession: NM_020581

